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Aluminium in allergen-specific subcutaneous immunotherapy – A German perspective

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ABSTRACT

We are living in an "aluminium age" with increasing bioavailability of the metal for approximately 125 years, contributing significantly to the aluminium body burden of humans. Over the course of life, aluminium accumulates and is stored predominantly in the lungs, bones, liver, kidneys and brain. The toxicity of aluminium in humans is briefly summarised, highlighting links and possible causal relationships between a high aluminium body burden and a number of neurological disorders and disease states.

Aluminium salts have been used as depot-adjuvants successfully in essential prophylactic vaccinations for almost 100 years, with a convincing positive benefit-risk assessment which remains unchanged.

However, allergen-specific immunotherapy commonly consists of administering a long-course programme of subcutaneous injections using preparations of relevant allergens. Regulatory authorities currently set aluminium limits for vaccines per dose, rather than per treatment course. Unlike prophylactic vaccinations, numerous injections with higher proportions of aluminium-adjuvant per injection are applied in subcutaneous immunotherapy (SCIT) and will significantly contribute to a higher cumulative life dose of aluminium. While the human body may cope robustly with a daily aluminium overload from the environment, regulatory cumulative threshold values in immunotherapy need further addressing. Based on the current literature, predisposing an individual to an unusually high level of aluminium, such as through subcutaneous immunotherapy, has the potential to form focal accumulations in the body with the propensity to exert forms of toxicity. Particularly in relation to longer-term health effects, the safety of aluminium adjuvants in immunotherapy remains unchallenged by health authorities – evoking the need for more consideration, guidance, and transparency on what is known and not known about its safety in long-course therapy and what measures can be taken to prevent or minimise its risks. The possibility of providing an effective means of measuring aluminium accumulation in patients undergoing long-term SCIT treatment as well as reducing their aluminium body burden is discussed.

1. Aluminium exposure

1.1. Aluminium in the environment

Aluminium (Al^{3+}) is the third most abundant element in the Earth's crust [1,2]. In 1825, it was isolated by the Danish physicist Hans Oersted [3]. Most aluminium is stably bound as an ore in clay, minerals, rocks and gemstones. Mobilisation of aluminium in the environment can result from natural processes (acidic precipitation) and through anthropogenic activities. This lightweight, non-magnetic, silvery white-coloured metal can be produced from the aluminium ore—bauxite—by a high energy-consuming mining process; it is this process which provides the world its main source of the metal. As a consequence of this technological progress, aluminium has become increasingly bioavailable for approximately the past 125 years [2]. Toxic mine tailings can leach and seep into aquifers, contaminating local water sources and soils. An increased solubility by anthropogenic pollutants such as acid rain is further contributing to this [5]. Most human exposure comes from the environment (the food we eat and the water we drink) [4]; additionally, aluminium is added for the coagulation of contaminants in drinking water. As a raw material, aluminium is used extensively in industry owing

Abbreviations: SCIT, Subcutaneous Immunotherapy; EFSA, The European Food Safety Authority; TWI, Tolerable Weekly Intake; CHMP, Committee for Medical Products for Human use; EMA, European Medicine Agency; PDCO, The Paediatric Committee; MPL, Monophosphoryl Lipid A; PEI, Paul-Ehrlich-Institut; DFG, German Research Foundation; WHO, World Health Organisation.

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to its unique and inherent properties (e.g. as a soft, light weight, resistant, non-corrosive metal). Aluminium and its compounds can be found in drinking water, our food, air, medicines, deodorants (antiperspirants), cosmetics and forms essential components in many household items and equipment, packaging, buildings and in aerospace engineering. It is the most widely used and distributed metal on the planet. Consequently, the human race is commonly referred to as living in an "aluminium age".

1.2. Human exposure to aluminium

Food, drinking water, air and medicines are considered to be sources of the aluminium load for humans (Fig. 1). With the utilisation of aluminium growing, bioavailability is increasing continuously. In 1950 this dietary aluminium load was thought to be approximately 1 mg per day, it is estimated to be 100 mg in 2050 [2]. Krewski et al. [4] present an overview of aluminium sources from foodstuffs and other products which contribute to this increase in exposure and subsequent load.

Uptake of Al^{3+} via the gastrointestinal tract is low: mostly reported as being between 0.1% and 1% [6], although considerably higher rates are described [7]. Of note, the bioavailability in drinking water is co-dependent on its silicic acid content: large amounts of silica in drinking water reduce the uptake of aluminium and vice versa [6,8]. Furthermore, aluminium interacting with various peptides, (glyco-) proteins and carbohydrates such as [iso-] citrate, malate, oxalate, succinate, tartrate, etc. must be taken into account. Such forms of aluminium significantly increase absorption rates [6,9–11].

Aluminium is excreted primarily via faeces and urine, with skin, hair, nails, sebum, semen, and sweat also having been described as excretion routes [2]. In fact, >95% aluminium is efficiently eliminated through the kidneys which helps explain why we can cope robustly with a daily dietary aluminium overload from the environment, minimising but not completely eliminating the risk of focal accumulations of the metal in other areas of the body. However, dialysis patients have been shown to bear levels of >30 μg/L aluminium in their sera, subsequently being linked with osteomalacia and related disorders [3]. High-risk individuals such as these would be at risk of longer-term health problems linked to aluminium accumulation/toxicity, outlined in Section 2 of this review.

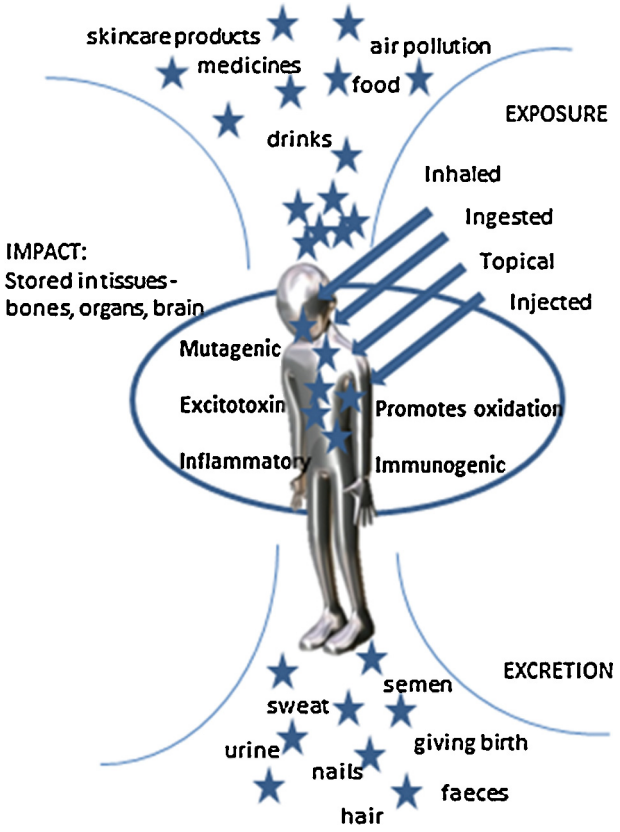

Fig. 1. A representation of human exposure to aluminium and its impact on the body [2].

Sweating particularly appears to be an underestimated excretion route for aluminium [12] that has been calling into question the widespread use of antiperspirants, which themselves contribute to the aluminium body burden [13,14].

Recently, the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung = BfR) calculated the daily systemic absorption of aluminium through the healthy skin to constitute 10.5 μg, which is above the amount considered safe for an adult (8.6 μg per day). Systemic absorption through damaged skin (e.g. after shaving) is much higher. The BfR therefore announced a warning not to apply an aluminium-containing antiperspirant shortly after shaving the armpit because of the significant contribution to the general aluminium body burden [15].

1.3. Aluminium in the body

Aluminium performs no obvious biological function in the human body and there is no evidence to date of aluminium-specific metabolism [16]. However, aluminium will take a number of different routes of absorption and interactions which will now be briefly summarised.

In the blood, >90% aluminium in plasma is associated with transferrin [2], with the approximate concentration of aluminium believed to be ~1–2 μg/L. The lungs and the bones are considered to be the major deposits in the body. Bone, lung, muscle, liver and brain are described as bearing approximately 60, 25, 10, 3 and 1% of the total body burden of aluminium, respectively [4]. Aluminium concentrations are also thought to increase with age [4]. The monocarboxylate transporter, the transferrin receptor shuttle, aluminium citrate and, recently described, ferritin are considered to be the transport routes of aluminium for crossing the blood–brain barrier [5,7–9,16]. In 2001, Yokel et al. published a half-life of 150 days of aluminium in the brains of rats following a single parenteral application of an ^{26}aluminium isotope [17].

Monitoring aluminium accumulation in humans is challenging. Urine and blood plasma analysis can be performed however neither will provide an accurate indication of the total aluminium body burden of an individual. Exley, 2013 best describes the true body burden of aluminium: "*for an individual is clearly not yet a quantity which is accessible by conventional means, at least not for a living person. While measurements of body burden are available these are actually indirect estimates of the systemic body burden, for example, the aluminium content of urine. These measurements are particularly helpful in comparing relative changes in the body burden of aluminium between individuals or between populations. They are, however, are less informative about where aluminium is found in the body or its potential for systemic toxicity*" [2].

1.4. Human threshold values

EFSA (The European Food Safety Authority) stated in a recent report [18]: "*in view of the cumulative nature of aluminium in the organism after dietary exposure, the Panel considered it more appropriate to establish a tolerable weekly intake (TWI) for aluminium rather than a tolerable daily intake (TDI)...*

...Based on combined evidence... the Panel established a TWI of 1 mg of aluminium/kg bw/week."

Animal studies are the rationale for the definition of this threshold value: *"The available studies have a number of limitations and do not allow any dose-response relationships to be established. The Panel therefore based its evaluation on the combined evidence from several studies in mice, rats and dogs that used dietary administration of aluminium compounds."*

In conclusion, the EFSA stated: *"The TWI of 1 mg/kg bw/week is therefore likely to be exceeded in a significant part of the European population. . ..*

. . ..Cereals and cereal products, vegetables, beverages and certain infant formulae appear to be the main contributors to the dietary aluminium exposure." [18]

In 2012, the WHO (World Health Organisation) defined a "*PTWI = provisional tolerable weekly intake*" of 2 mg/kg body weight as threshold and confirmed in the same document that this threshold is also achieved by adults consuming, e.g., cereals or, respectively, is exceeded regularly by children from the exposure to children's food [19].

The aluminium exposure of infants and toddlers from infant formulae appears to be particularly problematic. In a follow-up investigation by Chuchu and co-workers [20], commercially available formulae were again examined for aluminium. Regrettably, no reduction was found when compared to previous examination in 2010 [21]: the current aluminium concentrations in all 30 products examined were higher than the concentrations recommended for drinking water, 14/30 even exceeded the maximum allowable value of 200 μg/l [20]. Taking into account that at this age the blood–brain barrier has not fully matured, this (unnecessary) aluminium exposure appears complacent.

In summary, we have been living in a world with increasing bioavailability of aluminium for approximately 125 years, contributing significantly to the aluminium body burden of humans. The most common route of absorption with regard to volume is the gastrointestinal tract. Over the course of life, aluminium accumulates and is deposited predominantly in the lungs, bones, liver, kidneys and brain. While the human body may cope robustly with a daily aluminium overload from the environment, the regulatory cumulative threshold values in foods determined solely from animal studies are thought to be regularly exceeded. Any new or unnecessary additional exposures to aluminium have the propensity to overwhelm the body's coping mechanisms, with the potential to exert a form of toxicity. Of particular note are the forms of aluminium of pathophysiologic significance and associated longer-term health effects, which will be described and discussed in more detail.

2. Toxicity of aluminium in humans

Paracelsus: "All things are poison, and nothing is without poison; only the dose permits something not to be poisonous."

Aluminium has very well established neurotoxic properties. The most up-to-date and in-depth human health risk assessment of aluminium was conducted by Krewski and colleagues [4], who stated: "*Modest evidence of an effect exists for reproductive toxicity following oral exposure, for neurological toxicity following either oral or injection exposure, and for bone toxicity following injection exposure of aluminium*".

In the contemplation of toxicity, it is established practice to distinguish acute from chronic forms. This classification is reasonable for aluminium-related toxicity as well [6,22].

2.1. Acute toxicity

Acute toxicity refers to harmful effects caused by high concentrations of aluminium. Descriptions are available particularly with regard to dementia:

2.1.1. Dialysis dementia/dialysis encephalopathy

The first description of the aluminium-related dementias can be traced back into the 1970s [23,24] and most studies report a positive link between aluminium accumulation and cognitive impairments. However, some study designs are highly variable and their quality is questionable. More recently, evidence has demonstrated that high aluminium exposure from, i.e., drinking water can trigger acute episodes of dementia in patients with renal insufficiency, providing strong evidence for the causal relationship with aluminium [25].

The use of silicic acid has also been suggested to have a protective affect against the development of dementia [26–28]. As previously mentioned, the bioavailability of aluminium in drinking water is, for instance, co-dependent on its silica content: large amounts of silicic acid in drinking water reduce the uptake of aluminium and vice versa [6,10]. Exley and co-workers [26] have demonstrated that regular consumption of silicon-rich mineral waters reduce gastrointestinal uptake of aluminium and removal of systemic aluminium from the body. As a result, this may provide the basis of a non-invasive means for a therapy to treat the symptoms of Alzheimer's disease, in an attempt to reduce their body burden of aluminium. However, in-depth follow up studies involved in identifying clinical improvement of symptoms are at an early stage.

2.1.2. Aluminium powder in silicosis

In the 1940s, inhalation of aluminium was propagated as prophylaxis against silicosis in mine workers [29]. Examinations of these mine workers conducted in the study revealed the neurotoxic effects of this aluminium exposure [30].

2.1.3. Camelford accident

In 1988, the drinking water of the Camelford community in Cornwall, UK, was accidentally contaminated with 20 t of aluminium sulphate. Follow-up examination in the affected population demonstrated the consecutive neurotoxic effects of aluminium [31]. In another study, a neuropathological examination of an exposed individual who died from an unspecified neurological condition was performed. High aluminium levels were measured in affected regions of the cortex, where a rare form of β amyloid angiopathy was identified [32].

2.2. Chronic toxicity

Chronic toxicity refers to the harmful effects of protracted low-dose contamination.

2.2.1. Neurodegenerative effects

Increased concentrations of aluminium have been demonstrated in senile plaques in the brains of Alzheimer patients. The property of aluminium to produce amyloid-beta and cause damage to neurons, as well as epidemiologic connections, have been indicative of a relationship between aluminium and Alzheimer's disease for decades. Current reviews cite respective, but sometimes contradictory, studies [33]. To summarise the current state of knowledge, Bondy et al. [34], state "the final scientific proof of a causal relationship between aluminium and Alzheimer's disease is still pending. However, there is no longer any doubt about the neurotoxicity of aluminium in neurodegenerative diseases representing the chronic toxicity in humans".

In addition to these neurotoxic effects, a number of additional diseases, of which will be outlined, are being associated with aluminium as a causal relationship. However, the degree of evidence is somewhat weaker. Of note are:

2.2.2. Benign and malign diseases of the breast

A current review summarises the evidence on the relationship between aluminium and both benign and malignant diseases of the breast [14]. An increased absorption of aluminium from antiperspirants applied to the armpits is highlighted here. Such cutaneous absorption is increased by shaving the armpits, resulting in the recommendation not to apply deodorants immediately after shaving [15,35].

2.2.3. Myofasciitis

In France, a form of "macrophagic myofasciitis" is being discussed in connection with aluminium-containing adjuvants used in vaccinations that could trigger a cascade of immunological events associated with this autoimmune condition [36–39].

Additional diseases described are: autism [40], Gulf War Syndrome, allergies and other autoimmune diseases [41]. However, evidence here is poor and frequently the discussion is characterised by emotion.

In summary, though final scientific proof of a causal relationship between aluminium and Alzheimer's disease is still pending, there is no doubt about the neurotoxicity of aluminium. Predisposing an individual to an unnecessary high body burden of aluminium can be considered a prime cause for triggering toxicity linked to pathophysiologic significance.

3. Aluminium in prophylactic vaccinations

Aluminium compounds (e.g. aluminium oxyhydroxide; AlO(OH), aluminium phosphate; $AlPO_4$) have been used as adjuvants since 1926 [42,43], the exact mechanism of action is briefly summarised in Section 4.1.2 but it is not yet fully understood [44]. The vaccine preparation is primarily micrometer-sized clusters of nano-sized primary particles of the aluminium salt with which the antigen is associated with. The antigen physio-chemcial properties and form of aluminium will dictate the strength of adsorption [42]. There have been very few data reporting serious adverse reactions to aluminium in vaccines [45]. Aluminium salts are considered to be a stimulator of the Th2 immune response [44,46–50]. In addition to its adjuvant effects, they mediate a depot effect resulting in the antigen to be released more slowly from the injection site. It is inherent to this effect that aluminium salts when applied by the parenteral (usually intramuscular) route, stays in the body for prolonged periods of time.

Reflections on toxicity have resulted in ongoing and sometimes irrational discussion of the safety of aluminium-adjuvanted vaccines [41], which has the potential to invoke misguidance in the risk-benefit evaluations of immunisation programmes. Other investigations, such as Keith et al. [51], could not demonstrate a risk for infants examined. However, it is noted that the aluminium doses applied in vaccinations contribute to the lifelong human body burden of aluminium [46].

Currently the authorities do not conceive that aluminium-containing vaccines induce any potential (short- and/or long-term) hazards or safety issues. Since its first discovery by the English physician Edward Jenner, it is estimated that approximately 9 million lives have been saved as a consequence of vaccine immunisation, a significant proportion of which contain aluminium-based adjuvants [45]. Unlike most medications, essential vaccinations are given prophylactically to a healthy population (frequently children) in which the long-term benefits far outweigh any proposed risks, and form a pivotal component in the fight to eradicate disease.

The dose of aluminium salt in vaccines varies depending on the manufacturer; it could be as low as 170 μg per dose in Tripedia (diptheria/tetanus) or as high as 850 μg/dose in Tetramune (*Haemophilus influenzae* type b) [52]. It is important to take into account that the content of pure aluminium in e.g. AlO(OH) is approximately 45% (molecular weight of AlO(OH) = 60; aluminium = 27). Thus, based on the manufacturer's declaration, the proportion of aluminium in the AlO(OH) amounts to approximately half. Moreover, the number of prophylactic vaccinations against infectious diseases is usually low (e.g. up to three doses). A study by Keith et al. [51], calculated that exposure to aluminium from vaccinations in early childhood exceeds that from dietary sources, however, was calculated to fall below a minimal risk level set by The Agency for Toxic Substances and Disease Registry, U.S.

The design of double blind placebo controlled (DBPC) vaccination studies use (essentially toxic) aluminium adjuvants in placebo formulations, clearly adding unnecessarily to an individual's aluminium body burden. This anomaly makes it extremely difficult to assess the safety or risks of each study appropriately [53]. Furthermore, risk assessments frequently refer to the comparably, much higher environmental exposures to aluminium. The important differences between aluminium compounds that are applied parenterally or via the gastrointestinal tract are often negated [2]. This includes a difference in absorption (100% of aluminium absorbed via the parenteral route [17] versus 0.1–3% via the gastrointestinal route [see above]), and a prolonged clearance of such mediators of an aluminium depot effect is an inherent property of aluminium salts.

Despite the positive risk–benefit assessment of essential immunisation programmes, The French National Assembly published concerns in a summary of recommendations on vaccination, recognising the associated risks of aluminium accumulation and stated: *"In the light of the results of some studies carried out on aluminium. . ..it is necessary to research into new, non-neuromigrating adjuvants, which could eventually replace aluminium. . ."* [54].

In summary, aluminium salt compounds have been used successfully in essential vaccinations for almost 100 years, and the positive benefit-risk assessment remains unchanged. Aluminium-containing vaccinations against infectious diseases are adjuvanted with comparably low amounts of aluminium and are usually applied only a few times. Nevertheless, these amounts contribute to the cumulative overall human body burden of aluminium. In light of the growing number of toxicological considerations and as a tribute to the public discussion, research in aluminium-free vaccines should be encouraged and promoted.

4. Aluminium in SCIT

4.1.1. Principles and duration of SCIT

The prevalence of allergic disease is on the rise, it is estimated that almost half the population will develop some form of allergic disease during the course of their life. Allergen-specific immunotherapy commonly consists of administering subcutaneous injections using preparations of relevant allergens (Fig. 2), with the aim to gradually desensitise the allergic patient to the causative allergen. This may be achieved through the gradual release of natural/modified allergen extracts using a depot mediator (e.g. aluminium salts). By doing so, the natural course of the disease may be altered, being shown to redirect the immune response toward a Th1 immunoglobulin-type G profile and away from a predominant Th2 immunoglobulin-type E profile which is linked to the causative symptoms of allergy.

There are various regimens for SCIT treatment (Table 1) [55]. Usually, a phase of titration of the dose upwards is followed by a maintenance phase at a fixed dose. Some preparations allow for application intervals of up to 8 weeks, monthly injections are the recommended and customary practice.

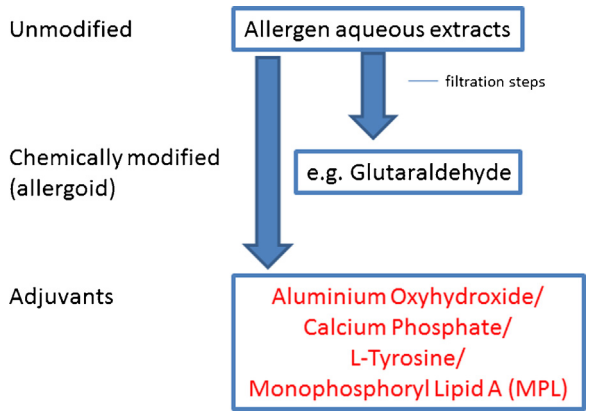

Fig. 2. Process flow outlining allergen-adjuvant preparation for immunotherapy [55].

For inhalant allergies, the specified therapy duration is 3 years with up to 5 years for house dust mite allergies [55]. SCIT is usually recommended for a duration of 5 years for hymenoptera venom allergies, whereas life-long monthly therapy may be given to sub-groups of patients who have an increased risk of more severe anaphylactic reactions. These sub-groups may have co-morbidities, or be prone to increased exposure (e.g. Bee-keepers) [56].

For a typical 3-year therapy, which would usually consist of, approximately 16 up-titration injections followed by monthly injections for a duration of 3 years, a patient will receive over 50 injections within this time-frame [57–59].

Five years of therapy as part of a house dust mite SCIT or hymenoptera venom allergy, >70 injections are administered in total [58]. Taking into account the subgroup of risk patients in hymenoptera venom allergy, the number of injections of this life-long immunotherapy rises infinitely.

Unlike the aforementioned vaccines, the manufacturers of SCIT products are not required to specify the amount of aluminium in their SmPCs (summary of product characteristics) or PIs (package leaflets). This is, however, in accordance to the German legislation = § 11 Arzneimittelgesetz (AMG). In Europe, 1.25 mg aluminium per injection is considered as the maximum value permitted [60]. The accumulative amount of aluminium during typical long-course SCIT is summarised in Table 2.

Upon subcutaneous injection, a local reaction forms once the antigen-adjuvant preparation comes into contact with the interstitial fluid (tissue space) and plasma. The majority of the adjuvant will remain in this vicinity for a number of hours, if not days. Dissolution of particulate aluminium will then occur, partly driven through a solubility/pH gradient. As more Al^{3+}(aq) evolves it then becomes available for binding by soluble ligands (e.g. transferrin and other proteins or ligands), thus accelerating the dissolution process [46].

The in vivo clearing of aluminium adjuvants has been studied in some detail using a radioactive isotope of aluminium (^{26}Al) administered in rabbits [63]. Mass spectrometry monitored the fate of the administered isotope for a period of 28 days. Approximately 1 h after injection, aluminium could be detected in the blood and remained steady for 28 days, however represented only a small fraction of the total aluminium dose administered. Urine samples monitored a 6% cumulative amount of aluminium eliminated in urine after 28 days, which was still being excreted. It must be stressed that neither such test will provide an accurate indication of the total systemic aluminium body burden of an individual and where it can be found in the body. However, in the same study the concentration of aluminium was approximately three times greater in tissues with the following distribution pattern: kidney > spleen > liver > heart > lymph node > brain.

As described in Exley [59], "*A single injection of 1 mg of aluminium adjuvant will add 1 mg of aluminium to the body burden but this milligram of aluminium will distribute throughout the body according to myriad different influences beginning with those occurring at the injection site*". While aluminium is released from the injection site and can be excreted, it clearly has the propensity to form small focal accumulations in body tissues (including the brain) which can arise and slowly build over the life-time of an individual.

Table 1
Definitions of sub-cutaneous allergy immunotherapy [55].

Short-term therapy	Approximately four to seven injections prior to the start of seasonal symptoms (e.g. hay fever).
Pre-seasonal therapy	Weekly injections are administered during a titration phase followed by monthly maintenance doses, prior to a pollen season.
Perennial therapy	Up to 16 weekly injections (titration phase) followed by monthly maintenance doses; reduction of the maintenance dose during the symptom season, if applicable.

Table 2
Long-term SCIT courses can significantly increase patient exposure to aluminium.

Product type	Aluminium allergy SCIT treatment [61,62]	Infectious disease vaccine (hepatitis B) [52]
Adjuvant	Aluminium oxyhydroxide	
Number of doses per course	36	3
Al content per dose (mg)	1.13	0.25
Cumulative Al dose (mg)	40.68	0.75

4.1.2. Aluminium salts: a stimulator of the Th2 immune response

The efficacy of aluminium compounds as adjuvants is undisputed, and similarly to vaccines they have been reportedly used in SCIT since 1937 [52]. The current guideline of German Allergy Societies classifies aluminium compounds as depot mediators [55]. Other commercial depot mediators used in SCIT are calcium phosphate and L-tyrosine. Although the gradual release explanation is inadequate to explain aluminium's adjuvant potential, the physical adsorption of antigen onto the adjuvant is still considered to be a very important mechanism. Particularly in SCIT where slower release of allergens from the injection site (thereby increasing the duration of antigen presentation) is pivotal in improving tolerability of the allergens [64].

However, aluminium adjuvants have been consistently demonstrated to induce IgE [44,46–50] which is clearly an unwanted and potentially adverse effect in any IgE-mediated disease, such as allergy. More recent mode-of-action studies have uncovered some aspects of how aluminium promotes a Th-2 response, but the precise role(s) of Th2-cytokines is not fully understood [44]. However, it appears that some this response may be mediated and signalled through a number of relevant interleukin pathways [44]. Since aluminium in SCIT is marketed and described as a depot adjuvant – a suitable depot carrier should support the immunogenic effect of specific immunotherapy without causing side effects. Aluminium salts have known side effects listed in the SmPCs, therefore physician–patient discussions form paramount importance in order to ascertain relevant risks. The incidence of persisting granulomas is reported to be 0.5–6% per hypersensitised patient, with the injection method being emphasised as a major factor affecting the frequency of the development of such granulomas [4]. Case reports describe local reactions, triggered by aluminium

compounds such as urticaria, subcutaneous sarcoidosis, progressive circumscribed sclerosis, formation of subcutaneous nodules and cutaneous–subcutaneous pseudolymphomas [4,6].

4.1.3. Aluminium accumulation in SCIT

Due to the evidence of the chronic toxicity of aluminium described earlier, the discussion of potential safety concerns in SCIT is not new [59,65]. The risk–benefit assessments of the national and international authorities have remained positive over the last number of years. This topic was addressed in detail in 2010 by the European Medicines Agency as part of the "*CHMP Safety Working Party response to the PDCO regarding Aluminium Hydroxide contained in Allergen Products*" [65]:

The Paediatric Committee (PDCO) of the European Medicines Agency (EMA) requested the EMA's Committee for Medical Products for Human use (CHMP) to provide a statement on the aluminium exposure with SCIT. The CHMP presented calculations on the annual cumulative aluminium dose applied in SCIT—for adults and children. Calculations were based on three scenarios: 1.14 mg, 0.5 mg and 0.15 mg aluminium per dose applied. The absorption rate was assumed to be 100% (*cf*. above). Six weeks were taken as a basis for application intervals during maintenance therapy. Thus, the authors calculated 9.12 mg, 4 mg and 1.2 mg aluminium, respectively, as cumulative absorbed annual dose in SCIT.

To compare the amounts of aluminium applied in SCIT, the CHMP's response to the PDCO indicated the "*real dietary intake (EU)*" and the "*safe oral dietary intake (TWI)*", respectively, for adults (65 kg) and for children (20 kg), with the statements of the EFSA and the WHO being used as the basis of the data—*cf*. above. The gastrointestinal absorption rate was based on the generally accepted range of 0.1–0.3%. Accordingly, the "*real dietary intake*" adds up to an annually absorbed amount of 0.7–15.4 mg and 0.73–7.2 mg in adults and children, respectively, and therefore clearly exceeds, particularly in children, the "*safe oral dietary intake (TWI)*" (3.3–10.1 mg and 1.0–3.1 mg in adults and children, respectively). This confirms the assumptions made by the EFSA and the WHO that the established thresholds are regularly exceeded, in particular in children—*cf*. above.

In addition, the CHMP based its assessment of chronic aluminium toxicity on pharmacovigilance databases (reports of serious and non-serious adverse events from the register of spontaneous reports or from clinical studies) from Germany from 1988 to 2008 (7638 reactions were analysed). Due to the low number of potential aluminium-associated side effects reported (except for the known granulomas), the CHMP arrived at the conclusion that there are no safety concerns. To what extent such a database is suitable to detect associations between SCIT and the development of diseases, which could have a latency period, remains to be seen.

In their conclusion, the Safety Working Party to the CHMP places the cumulative aluminium dose of 12 mg aluminium absorbed from a 3-year SCIT (0.5 mg per injection, 6-week interval = 4 mg per year × 3 years of therapy) in the context of an adult's lifelong cumulative dose of 165–505 mg as "*safe oral dietary intake (TWI)*". Thus, the contribution of such an SCIT to the lifelong cumulative total dose is calculated as being fewer than 10%. In connection with the estimation on the basis of the side effects database, the CHMP draws the conclusion that there is no risk from aluminium in SCIT [65].

4.1.4. The underlying assumptions under close scrutinisation

It is general practice in toxicology to consider maximal values (within a licensed indication) of the substance in question. The final assessment of the CHMP does not seem to be based on a similar rationale and it ignored up-titration period(s) completely. If 1.14 mg (top aluminium-adjuvant dose) is considered and 6-week intervals, then the human body burden of aluminium totals 27.36 mg (1.14 mg × 8 × 3 years). If the maintenance dose were based on monthly (*cf*. above) instead of the 6-week intervals, this amounts to 41.04 mg (1.14 mg × 12 × 3 years) and still would not include up-titration. Over the course of their lives, many allergic patients will receive treatments for several allergens—some lifelong (*cf*. above). The cumulative dose of aluminium from immunotherapy used as basis by the CHMP does not appear to reflect the amount of exposure a patient will receive in practice. In addition to this, it was compared to dietary intake (i.e. the immunotherapy cumulative dose being <10% of this) – a route of administration with a totally different adsorption rate. This is not only misleading but a fundamental mistake.

In January 2014 the Paul-Ehrlich-Institut (PEI) published its opinion regarding aluminium in SCIT "Sicherheitsbewertung von Aluminium in Therapieallergenen" [66]. Within this document, the German regulatory authority essentially repeats conclusions drawn from the CHMP in 2010 [65]. For the general risk assessment of aluminium PEI refers to the above-mentioned EFSA statement and a risk assessment from the German Bundesinstitut für Risikobewertung (BfR) from 2007 [15]. Pharmacovigilance (PhV) databases were screened from 1986 until 2013 without revealing signals – though as highlighted above there are doubts about as to the use of such a database in uncovering relationships between SCIT and e.g., neurodegenerative diseases having a latency period of many years. A perceived *positive* benefit–risk-ratio is reiterated in their statement.

However, since the potential of accumulation of aluminium in the body is clearly significant in the course of SCIT, companies themselves indicate in relevant sections of their SmPCs as follows:

> *"During therapy with AVANZ® preparations, taking aluminium-containing drugs (e.g. antacids) should be restricted."* [67].

Additionally, *"This product contains aluminium (4 mg). The risk of aluminium accumulation in tissues (CNS, bones) must be taken into account, in particular in case of renal insufficiency. The effects on the immune system of long-term administration of aluminium are unknown. As this preparation contains a considerable amount of aluminium, it is recommended to avoid taking other aluminium-containing medications (*e.g. *antacids) concomitantly."* [68].

Furthermore, *"Patients with Alzheimer's disease, Down's syndrome and renal insufficiency are theoretically at risk from aluminium intake, including alum precipitated allergenic extracts"* [69].

4.1.5. Aluminium as an antigen

While so far it has not yet been definitely clarified which form of aluminium acts as an antigen [70], immune reactions to antigenic aluminium as a consequence of SCIT is plausible. Such immune reactions would target aluminium deposits in the human body, which has the potential to contribute to the onset and progression of aluminium-induced autoimmune diseases [59].

4.1.6. Alternatives to aluminium

The amount of aluminium in SCIT is a significant addition to the lifelong exposure to the metal in children and adults. Taking this into account the toxicological considerations, it is not unreasonable to question the long-term impact this has on human health. Long-term aluminium adjuvant-based immunotherapy treatment unquestionably predisposes an individual to a likely set of circumstances that could lead to accumulation, toxicity and disease.

According to Good Pharmacovigilance Practices, assessment of a benefit–risk relation must take into account the severity of the treated disease (e.g. hay fever), the presence of therapy alternatives,

Table 3
Overview of typical detection methods for measuring aluminium in biological samples [76].

	Flame AAS	GF-AAS	ICP AES	ICP-MS
Method description	The sample is dissociated through the application of an air/acetylene or a nitrous oxide/acetylene flame. Atoms will absorb the light from a cathode lamp and a detector is used to measure the concentration(s).	Identical principle to FAAS but using an electrically heated graphite tube, or cuvette, which is heated to a temperature up to 3000 °C to dissociate the sample.	Uses an inductively-coupled plasma source to dissociate the sample, atoms/ions are excited and their characteristic wavelength is then measured.	Uses an inductively-coupled plasma source to dissociate the sample, atoms/ions are detected directly as opposed to measuring their excited wavelength emission(s).
Detection range	30 ppb	0.25 ppb	1.5–6 ppb	0.1–10 ppt
Strengths	Ease of use, sampling speed, value for money, robust interface and compact instrument.	Sensitive detection limit, moderate cost, compact instrument with low risk of spectral interferences	Ease of use, expensive but cost-effective in relation to multi-element sampling and screening ability. Solid and organic samples, low-risk of chemical interferences.	Most sensitive detection limit, expensive but cost-effective in relation to multi-element sampling and screening ability, isotopic measurements, lower risk of interferences, output is easy to interpret.
Limitations	Least sensitive detection limit, limited number of elements, and no screening ability.	Higher risk of chemical interferences, element limits, lack of screening ability.	Higher risk of spectral interferences with multi-elements and sample types, detection limits can be moderate.	Highly skilled operator/training required, expensive, dissolved solids must be <0.2%.

and to the type of risk assessed. In Germany, licensed and comprehensively documented alternative products with other depot mediators are commercially available for example use of L-tyrosine, a non-essential amino acid physiologically generated from phenylalanine and fully metabolised with a half-time of 48 h, has been well-documented as a commercial alternative for over 40 years [71–73]. Other endogenous and biodegradable adjuvant systems are researched but difficulties in achieving regulatory approval without having extensive mode-of-action and safety studies, making it costly and time-consuming to bring market [74]. TLRToll-like receptor agonists use in immunotherapy (e.g. MPL/CpG motifs) has shown some excellent benefits [64]. However, such adjuvants will not function as depot mediators. The physical adsorption of antigen onto the adjuvant and subsequent 'slow-release' of antigen is considered to be a very important mechanism, particularly in SCIT. In some products, the depot mediator – L-Tyrosine – is used in combination with MPL. Here, Tyrosine allows slow release of allergens. While MPL will drive an appropriate immunological response (Th1), thus enabling a unique ultra-short course therapy for the allergic patient [75].

In summary, the amount of aluminium applied in SCIT will significantly contribute to a higher cumulative life dose. Unlike essential prophylactic vaccinations, numerous injections with higher proportions of aluminium-adjuvant per injection are applied in SCIT. Comparably high amounts of aluminium are administered, particularly during long-term SCIT for hymenoptera venom allergies whilst there are aluminium-free products commercially available.

5. Aluminium analysis

Aluminium analysis is technologically demanding. The very low concentrations and possibility of contamination poses problems. Aluminium compounds are of biological significance—*cf.* above. The stability of these aluminium compounds constitutes an additional complicating factor in analysis.

However, several methods are available:

The atomic absorption spectrometry (AAS), and particularly graphite furnace atomic absorption spectrometry (GF-AAS), are single element methods with detection thresholds of approximately 1 μg/L. This method is commonly applied for analysing biological samples and aqueous media. However, inductively coupled plasma–optical emission spectrometry (ICP-OES) now provides a more sensitive alternative, able to measure lower concentrations of the metal, especially when using quadrupol (ICP-qMS) or high-resolution sector field ICP-MS (ICP-sf-MS). These devices are however expensive and of limited availability. Table 3 summarises the type of analytical methods mentioned above, their detection range(s), strengths and limitations.

The German Research Foundation (DFG) assembled an independent expert group entitled "Analyses in Biological Material". This group has published research papers on threshold values and methods (MAK collection) and are able to advise on how to reasonably measure, e.g., the aluminium exposure caused by SCIT [77]. There is currently no generally accepted surrogate parameter which would reflect the cumulative burden to the body posed by aluminium [19].

In summary, aluminium analysis is expensive and highly demanding although the technology is available to detect trace amounts of the metal in biological samples. The DFG provides independent expertise with the work group "Analyses in biological material". It is important to note that blood and serum analysis of aluminium provide unreliable indications in relation to an individual's body burden of aluminium. There is currently ongoing work on ways in which to measure aluminium accumulation in humans via non-invasive means. As previously described, one such method utilising silica-enriched water has thus far yielded promising results and has been shown to reduce the human body burden of aluminium. Currently, this method has been shown to reduce the body burden of aluminium in Alzheimer's patients, and release systemic aluminium in urine [26,28]. Its application in other contexts such as in patients undergoing long-term SCIT treatment could be similarly applied.

6. Summary

Anthropogenic factors over the past 125 hundred years have increased human exposure to aluminium, resulting in a burgeoning body burden of this neurotoxin.

Threshold values for foodstuffs established by authorities are regularly exceeded and aluminium compounds are regularly used as adjuvants in vaccinations. In SCIT, aluminium compounds are employed as adjuvants and depot mediators. Unlike essential prophylactic vaccinations, numerous injections with significantly higher proportions aluminium per injection are applied during SCIT. However, regulatory authorities currently set aluminium limits for vaccines per dose, rather than per treatment course.

Based on the currently available literature, the benefit–risk relationship of long-term aluminium adjuvant SCIT should be re-assessed according to Good Pharmacovigilance Practices. Aluminium will accumulate in the human body over the life-time of an individual and undoubtedly has the potential to exert chronic toxic effects, such as neurotoxicity. Predisposing an individual to an unnecessary high body burden of aluminium should be avoided and could reasonably be considered a cause for triggering the onset or progression of a number of conditions and disease states mentioned in this paper. There is however still a lack of epidemiological studies examining the possible relationship between the developments of such diseases, which may have a latency period of many years after the application of SCIT.

In currently on-going SCIT studies, aluminium accumulation should be more accurately measured for the entire treatment period. External expertise as provided by the DFG should be collected for planning such bio-monitoring. There is currently on-going work, using silica-enriched water, to measure aluminium accumulation in humans via non-invasive means and ascertain more accurate indications of an individual's body burden of aluminium. This could open up the possibility of providing an effective means of measurement in patients undergoing long-term SCIT treatment, as well as reducing the aluminium body burden.

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Conflicts of interest. Prof. Dr. med. Matthias F. Kramer is the International Medical Director of Allergy Therapeutics plc. Dr. Matthew D. Heath, PhD, is a Lead Medical Writer at Allergy Therapeutics plc. Allergy Therapeutics market aluminium-free SCIT products.

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